IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

Attorney Docket No. 065691/0222

In re patent application of

Pierre CHAMBON *et al.*

Serial No. 09/853,033 Group Art Unit: 1636

Filed: May 11, 2001 Examiner: Celine X. QIAN

Title: TRANSGENIC MOUSE FOR TARGETED RECOMBINATION MEDIATED BY MODIFIED CRE-ER

DECLARATION UNDER 37 CFR § 1.132

Assistant Commissioner for Patents
PO Box 1450
Alexandria, VA 22313-1450

I, Pierre H. Chambon, hereby declare that:

1. I am a co-inventor on the above-captioned application. I have held the position of Director of the Institut Clinique de la Souris since 2002. My qualifications are set out in my *curriculum vitae*, which is attached hereto as APPENDIX A.

2. Many genes and proteins encoded by these genes are expressed and function in different cell types and at different stages of development and adult life, and may be implicated in different pathological states. Therefore, an important tool for understanding the function of given genes in different processes would be the ability to delete or modify a given gene at will in a given tissue during a particular stage of development or adult life in whole animal models.

3. The mouse is currently considered the most appropriate mammalian model because of similarities between mouse and human genomes, the amount of mouse genetic and biochemical data available, the relative ease of maintaining experimental mouse colonies, and the experimental tools that have been developed for the mouse.

4. The present invention provides a method for efficient life stage- and tissue-specific modification of a given endogenous gene in its natural genomic environment in the living mouse. At the time of filing, examples of such modifications in three cell

populations (keratinocytes, adipocytes and hepatocytes), from three distinct organs, using different transgenic mice expressing fusion proteins were provided. Each of these fusion proteins contained a modified estrogen receptor ligand binding domain (ER^T, ER^{T2}) conferring in vivo tamoxifen-inducibility on the activity of the fused Cre recombinase. These transgenic lines were then bred with mice bearing endogenous genes, in their normal chromosomal position and environment, that contained engineered LoxP sites specifically recognized by Cre recombinase. Thus, upon tamoxifen treatment, the fused Cre recombinase specifically deleted, in the progeny, gene segments flanked by LoxP-sites ("floxed segments).

5. In each case, the deletion of the "floxed" segment was 100% efficient following tamoxifen treatment in all cells in which the recombinase was expressed. Moreover, a deletion was not observed in the absence of tamoxifen, thus demonstrating that the inventive method permitted tight temporal control of the generation of cell type- or tissue-specific somatic mutations with a 100 % efficiency.

6. Clearly, achieving in the mouse, with a 100 % efficiency, tightly temporally-controlled somatic mutations that are targeted to endogenous genes in their normal chromosomal position and environment, was not obvious to one of ordinary skill in the art at the time of filing from published work on Cre-ER fusion proteins. Indeed :

   (a) the work of Feil *et al.* (Proc. Natl. Acad. Sci. USA 98, 10887-10890, 1996) and Indra *et al.* (Nucl. Acid. Res. 27, 4324-4327, 1999) had only demonstrated that Cre-ER^T or Cre-ER^{T2} fusion proteins could be used to efficiently delete "floxed" DNA segments located within exogenous synthetic reporter transgenes.

   (b) Schwenk *et al.* (Nucl. Acid. Res. 26, 1427-1432, 1998) had demonstrated that a tamoxifen-inducible Cre-ER fusion protein could be used to delete a "floxed" DNA segment from an endogenous chromosomal gene, but importantly, the excision efficiency was variable and incomplete. High doses of tamoxifen had to be used to reach at best an 80% deletion efficiency.

This Schwenk et al. failure to achieve with a 100% efficiency the excision of a "floxed" DNA segment from endogenous chromosomal genes clearly showed that one could not anticipate the efficiency of the Cre-ER^{T} and Cre-ER^{T2} recombinases to excise DNA segments belonging to chromosomal genes that are located in their physiological environment, from their efficiency to excise DNA segments located in exogenous reporter genes, as Schwenk et al. had reported in the same paper a 100 % efficiency of excision for a "floxed" DNA segment from an exogenous reporter gene in mouse embryonic fibroblasts.

Thus, even though Feil et al. and Indra et al. taught us that Cre-ER^{T} and Cre-ER^{T2} recombinases resulted in efficient Cre-mediated excision of a floxed DNA segment from synthetic reporter genes, it was definitely not obvious to one of ordinary skill in the art to expect successful complete excision of sequences within endogenous genes located in the mouse in different chromosomal environments.

7. Since the time of filing, I and my collaborators, have constructed additional mouse lines that express tamoxifen-activated Cre-ER^{T} and Cre-ER^{T2} fusion proteins in a variety of cell types/tissues, and allow efficient tamoxifen-dependent spatio-temporally-controlled targeted somatic mutagenesis in the mouse, confirming the generality of the present invention.

The general nature of our Cre-ER^{T2} system is also supported by its present worldwide use to excise "floxed" DNA segments from various target chromosomal genes in a variety of tissues or cell types. For instance, efficient hepatocyte-selective ablation of a "floxed" HNF4 gene was recently obtained by Dr. F. Gonzalez (Ph.D., Laboratory of Metabolism, Center for Cancer Research, National Cancer Institute, Bethesda, USA), using our Cre-ER^{T2} system. Dr. F. Gonzalez wrote us :

"The albumin Cre-ER^{T2} mouse is working great with complete knockout of HNF4 in the liver after 4OH-T administration".

8. I hereby declare further that all statements made herein of my own knowledge are true and that all statements made on information and belief are believed to be true and

further that these statements were made with the knowledge that willful false statements and the like so made are punishable by fine or imprisonment, or both, under Section 1001 of Title 18 of the United States Code and that such willful false statements may jeopardize the validity of the application or any patent issuing thereon.

By: _____
Pierre H. Chambon, M.D.

Date: 13 October 2004